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Growth and yield response of *Cuphea* to row spacing

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Abstract

Presently, in USA there is no domestic crop source of oil rich in medium chain fatty acids (MCFAs) (i.e., oils composed of triglycerides with fatty acid chains between 8 and 14 carbons long). Several species of the genus *Cuphea* (Lythraceae) accumulate saturated MCFAs in their seed-storage lipids, and some species grow well in short-season temperate climates. Recent efforts have been successful in semi-domesticating genotypes of *Cuphea* for crop production. However, little is known about best management practices for production. A 2-year study was conducted in west central Minnesota, USA, to determine optimum inter-row spacing for row culture production of *Cuphea*. Seed was sown in inter-row spacings of 0.125, 0.25, 0.375 and 0.50 m in 1999, and a fifth spacing of 0.75 m was added in 2000. In 2000, seed yield averaged nearly 1000 kg ha^{-1}, which was about 40% greater than in 1999. Seed yields were not significantly affected by row spacing. Plants in wider rows compensated for yield by producing more branches and seed pods per plant. The number of filled pods per plant was as much as 70% greater for plants in the widest as compared to the narrowest row spacing. Data indicate that this was due in part to competition among plants for available light and nutrient resources. Row culture of *Cuphea* in the northern Corn Belt of the US appears favorable. However, due to its indeterminate habit, growth and yield of *Cuphea* in row culture may be more responsive to plant population density than inter-row spacing.

Keywords: Row spacing; Seed yield; Growth; *Cuphea*

1. Introduction

Medium chain fatty acids (MCFAs), those ranging from 8 to 14 carbon chain lengths, are highly valued as feed-stocks in the chemical manufacturing industry. They are used to make a wide variety of products including soaps and detergents, personal-care products, nutritional and dietetic products, lubricants and related products (Thompson, 1984). Sources of MCFA used in the United States are petrochemicals, and coconut (*Cocos nucifera* L.) and palm kernel (*Elaeis guineensis* Jacq.) oils. Presently, there is no domestic source for these oils. Between 1995 and 2000, the United States alone imported an average 644,165 metric tons of coconut and palm kernel oil per year at a yearly mean cost of about $ 450 million (United Nations FAO, 2002) to meet chemical manufacturing demands for MCFA. Furthermore, ‘‘developed’’ countries together imported

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2.1 million metric tons per year during this same period (United Nations FAO, 2002).

During the 1960s, the genus *Cuphea* (Lythraceae) was found to contain many species whose seeds are rich in MCFA (Miller et al., 1964). Analysis of the seed oil of several *Cuphea* species has shown that many emphasize the production of one or a few fatty acids in the range of C8:0 to C14:0 (Graham, 1989; Graham and Kleiman, 1992). Of about 260 species of *Cuphea* that have been identified, several are summer annuals that are widely adapted to temperate climates (Graham, 1989). Recently, a semi-domesticated genotype (PSR23) was shown to have a similar cropping season to that of soybean produced in the northern Corn Belt (Gesch et al., 2002).

Hirsinger (1985) evaluated 30 different *Cuphea* species and found several belonging to the Heterodon section (Koehne, 1903) that showed favorable agronomic traits, making them potential candidates for domestication. Across 21 species in the Heterodon section that were tested, the average seed yield per plant was 4.3 g. Hirsinger's study was conducted in the field (near Davis, CA); however, no details were given about the cultural methods used. Assuming a plant population of 440,000 ha^{-1}, similar to that used for soybean production, then a seed yield of 4.3 g per plant would theoretically produce 1892 kg ha^{-1}. *Cuphea wrightii* was shown to produce 6.0 g of seed per plant, but when field-cultivated, yielded a maximum of 900 kg ha^{-1} (Hirsinger, 1985). However, Hirsinger noted that production practices were not optimized for the field experiment. These results obviously indicate a large disparity between yield on a per plant and land area basis.

Commercial production of *Cuphea* has been hindered by agronomically unfavorable wild-type traits, particularly seed shattering, seed dormancy, and self-incompatibility (Knapp, 1990). However, progress over the past decade towards domesticating *Cuphea* as a commercial source of MCFAs has led to the development of germplasm lines that are self-compatible, partially non-shattering, and non-dormant (Knapp, 1993). One such line is PSR23, which was developed from an interspecific hybridization of *C. viscosissima* and *C. lanceolata* (Knapp and Crane, 2000). It is a dicotyledonous, herbaceous, summer annual with an indeterminate growth habit, and is self-compatible though strongly cross-pollinated (Knapp and Crane, 2000).

To realize the full yield potential of *Cuphea*, agricultural practices will have to be optimized for its production. There are no known reports identifying the effects of inter-plant spacing on *Cuphea* growth and seed yield. The objectives of the present experiment were to determine the effects of row spacing on seed yield and growth of *Cuphea* plants. Row culture was chosen as opposed to solid-stand methods because row-cropping, which allows for mechanical weed control, is the predominant practice in the northern Corn Belt and effective chemical weed control for *Cuphea* is lacking.

2. Materials and methods

Experiments were conducted in 1999 and 2000 at the Swan Lake Research Farm located 24 km NNE of Morris, MN (45°40′N) on an Sverdrup sandy loam soil (coarse-loamy, mixed, Udic Haploboroll). *Cuphea* (*Cuphea viscosissima* Jacq. × *C. lanceolata* f. *silenoides* W.T. Aiton, PSR23) was sown by hand at a 1 cm depth in rows spaced 0.125, 0.25, 0.375, and 0.50 m apart in 1999 and a 0.75 m spacing was added in 2000. Plots were constructed in a randomized complete block design with three replications. For the 0.125 m spacing, plots consisted of five 1 m rows, while three 1 m rows were planted for the remaining treatments. Due to the small quantity of *Cuphea* seed that was initially available for the study, a row of soybean was grown adjacent to the outside rows of each plot at the given treatment spacing and pruned to the same height as *Cuphea* to reduce boarder effects. Seed weight of PSR23 is about 2.7 mg (Gesch et al., 2002). Because of low germination rate of the seed-lot received (24% at 25 °C, data not shown), plot rows were sown relatively heavily and were not thinned after plants emerged. Seed was sown at a rate of 0.5, 1.0, 1.5, 2.0 and 2.0 g m^{-1} of row for the 0.125, 0.25, 0.375, 0.50 and 0.75 m row spacings, respectively. Plots were sown on 1 June 1999 and 15 May 2000 and were periodically watered until plants emerged. In 1999, plots were fertilized with 80, 76 and 72 kg ha^{-1} of nitrogen, phosphorus, and potassium, respectively. The fertilizer was applied in soluble form and split into five applications at approximately weekly intervals beginning at emergence. In 2000, 112, 13 and 30 kg ha^{-1} of N–P–K were incorporated at one time into the upper 0.15 m of soil prior to sowing. All plots were hand-weeded.

All rows for each plot were hand-harvested at 1011 GDD (°C days, using 10 °C as the base temperature) in 1999 and 1034 GDD in 2000 from the time of planting. The harvest dates for 1999 and 2000 were 8 and 6 September, respectively. The criterion used for time of harvest of this indeterminate crop was based on observations of when the most mature seed pods began to shatter. Seed pods were separated from plants and all plant material was air-dried in a greenhouse for 21 days. After drying, biomass was weighed and seed was threshed and screen-cleaned by hand. Stand counts were taken at harvest.

Immediately before harvest, three plants per plot were randomly sampled for analysis of growth and yield components. Plants for this analysis were clipped at the soil surface and placed in a large cooler for transporting to the laboratory. Leaf area was measured with a leaf area meter (LI-3100,[1] Li-Cor, Lincoln, NE) within 120 min after clipping. Branches were counted if they were $\geq$0.2 m. Dry weight of plant material was determined after drying in a forced air oven at 65 °C for 60 h.

During the 2000 growing season, leaf area index (LAI) was measured at first flower on 19 July and during seed filling on 16 August. Measurements were taken while skies were overcast between 1400 and 1600 h Central Standard Time with an LAI-2000 LAI meter (Li-Cor, Lincoln, NE) at two locations in each plot using a 45° angle reduction cap. At each location within a plot, four individual measurements were made in a diagonal pattern between rows and averaged.

Cuphea seed oil content was determined by pulsed NMR using procedures described by Gesch et al. (2002). Before use, the instrument was calibrated using known amounts of extracted oil from PSR23 *Cuphea* seed. Total nitrogen and carbon were determined for two 0.4 g sub-samples of seed from each plot using a Leco CN-2000 combustion device (Leco, St. Joseph, MI). Values of total seed oil, nitrogen, and carbon are reported on a weight/weight basis (g per kg of air-dried seed).

Cuphea seed and total biomass yield were evaluated for each year separately using the GLM procedure of SAS for analysis of covariance (SAS Institute, Cary, NC) with plant population as the covariate and row spacing as the main effect. All other data were analyzed for each year separately using the ANOVA procedure of SAS. Least significant differences (LSDs) at the $P = 0.1$ level were used to detect differences between least-squares means.

3. Results and discussion

Despite increasing seeding rate with row spacing, plant densities significantly declined with wider rows in both years (Table 1). The mean intra-row spacing differed by 1.6 cm between the widest and narrowest row spacing in both years (Table 1). The mean area occupied per plant was 0.0044 and 0.0095 m^2 for the narrowest and widest row spacings, respectively, in 1999 and was 0.0036 and 0.0098 m^2, respectively, in 2000. Since plant density is a determinant of yield, an analysis of covariance was used to evaluate total biomass and seed yield. Generally, seed yields were greater in 2000 than they were in 1999. This was likely due to either the earlier planting date in 2000, as early to mid-May has been shown to be an optimal planting time for *Cuphea* (Gesch et al., 2002), or due to the different fertilizer treatment between years. Nevertheless, trends were similar in both years. In neither year did row spacing have a significant effect on seed yield (Table 1) when means were adjusted using plant density as a covariate. Likewise, total biomass was not affected significantly by row spacing in 1999, although in 2000 it was significantly higher ($P = 0.1$) for the 0.25 m spacing in comparison to the other treatments (Table 1). Seed size, as assessed by 1000-seed weight, was uniform across treatments (Table 1).

Seed oil content was slightly lower in 1999 (258 g kg^{-1}) than 2000 (279 g kg^{-1}) (Table 2). These values are similar to those previously reported for *Cuphea* grown in west central Minnesota, USA (Gesch et al., 2002), but slightly less than 295 g kg^{-1} reported by others for PSR23 (Knapp and Crane, 2000). Variation in seed oil content between years might have been due to differences in sowing date. Gesch et al. (2002) showed that sowing date can influence *Cuphea* seed oil content. Differences in

[1] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

Table 1
Effect of row spacing on *Cuphea* total biomass and seed yield for 1999 and 2000[a]

Year	Inter-row spacing (m)	Intra-row spacing[b] (cm)	Plant density (10^6 plant ha^{-1})	Total biomass yield[c] (DW Mg ha^{-1})	Seed yield (kg ha^{-1})	1000-Seed weight (g)
1999	0.125	3.5 ± 0.1	2.28 a	8.2 a	350 a	2.69 a
	0.250	2.2 ± 0.2	1.86 b	9.5 a	526 a	2.98 a
	0.375	2.0 ± 0.3	1.39 c	9.4 a	636 a	2.68 a
	0.500	1.9 ± 0.5	1.18 d	9.7 a	705 a	2.59 a
Mean		2.4	1.68	9.2	554	2.74
2000	0.125	2.9 ± 0.7	3.01 a	15.1 b	933 a	2.94 b
	0.250	1.9 ± 0.2	2.18 b	17.2 a	940 a	2.97 b
	0.375	1.6 ± 0.3	1.78 bc	15.2 b	898 a	2.92 b
	0.500	1.3 ± 0.1	1.59 cd	14.8 b	1013 a	2.95 b
	0.750	1.3 ± 0.1	1.03 d	14.7 b	1083 a	3.09 a
Mean		1.8	1.92	15.4	973	2.97

[a] Mean values within columns by year followed by the same letter were not significantly different at the $P = 0.1$ level.
[b] Mean values ± S.E.; calculations were based on number of plants per meter-row for all rows per plot.
[c] Total biomass and seed yield were evaluated using analysis of covariance using plant density as the covariate. Values are the adjusted means.

fertilizer management may also have been a contributing factor. Nitrogen content of seed was on average greater in 2000 than in 1999 (Table 2). Furthermore, in 2000 there was a trend, albeit weak, of increasing nitrogen content concomitant with a decrease in oil content as row spacing increased (Table 2). This change in nitrogen content with row spacing is indicative of nutrient competition. As row spacing decreased, plant population density increased, which might have caused greater competition for available soil nutrients, thus leading to lower nitrogen levels. The mean seed carbon content was identical between years with little difference across treatments (Table 2).

Table 2
Effect of row spacing on seed quality of *Cuphea* for 1999 and 2000[a]

Year	Row spacing (m)	Oil content (g kg^{-1})	Nitrogen content (g kg^{-1})	Carbon content (g kg^{-1})
1999	0.125	274 a	31.0 a	542 a
	0.250	274 a	31.5 a	551 a
	0.375	239 a	31.9 a	540 a
	0.500	244 a	31.9 a	541 a
Mean		258	31.6	541
2000	0.125	281 abc	32.6 b	540 ab
	0.250	289 a	32.6 b	542 a
	0.375	283 ab	33.9 ab	543 a
	0.500	273 bc	34.3 ab	537 b
	0.750	270 c	35.0 a	543 a
Mean		279	33.7	541

[a] Values of total seed oil, nitrogen, and carbon are reported on a weight/weight basis (g per kg of air-dried seed). Mean values within columns by year followed by the same letter were not significantly different at the $P = 0.1$ level.

Characteristics of *Cuphea* plants grown in different row spacing are shown in Table 3. Growth of *Cuphea* in rows spaced 0.50 m apart or greater consistently led to larger plants. However, there was no clear effect of row spacing on plant height (Table 3). Rather, the increase in plant size was primarily due to increased branching with wider row spacing. Increased branching of plants sown in rows spaced greater than 0.375 m apart resulted in generally greater leaf area, number of filled pods, and seed weight per plant (Table 3).

Cuphea produces flowers on branches extending from main stem nodes as well as the main stem itself (Graham, 1989). It has an indeterminate growth habit and once flowering begins it can continue for up to 2 months (Hirsinger, 1985; Graham, 1989). The data presented in Table 3 indicate that *Cuphea* grown in rows spaced 0.50 and 0.75 m compensated for yield by increasing the number of filled pods and hence seed per plant. Indeed, when the number of filled pods per square meter area was analyzed, taking into account plant density, there was not a significant difference

Table 3
Effect of row spacing on growth characteristics of *Cuphea* for 1999 and 2000[a]

Year	Row spacing (m)	Aboveground biomass (DW g per plant)	Height (cm per plant)	Number of branches[b] (per plant)	Leaf area (dm^2 per plant)	Number of filled pods (per plant)	Seed yield (g per plant)
1999	0.125	5.2 b	98.1 ab	4.8 ab	4.0 b	15.8 b	0.23 b
	0.250	5.4 b	101.6 a	3.2 b	5.0 ab	16.4 b	0.32 b
	0.375	7.4 a	95.5 b	6.9 a	5.8 ab	33.0 a	0.58 a
	0.500	8.7 a	100.8 ab	7.7 a	6.2 a	36.6 a	0.63 a
Mean		6.7	99.0	5.7	5.3	25.5	0.44
2000	0.125	5.1 b	104.8 ab	2.1 b	5.8 c	19.3 c	0.34 c
	0.250	6.3 b	108.3 a	4.9 b	8.1 bc	32.6 bc	0.50 c
	0.375	6.7 b	104.2 ab	3.3 b	7.9 bc	28.0 c	0.43 c
	0.500	10.2 a	106.7 ab	9.4 a	10.7 ab	50.0 ab	0.86 b
	0.750	12.3 a	98.3 b	8.9 a	12.1 a	64.4 a	1.20 a
Mean		8.1	104.4	5.7	8.9	38.9	0.67

[a] Mean values within columns by year followed by the same letter were not significantly different at the $P = 0.1$ level.
[b] Branches were counted if they were ≥ 20.0 cm.

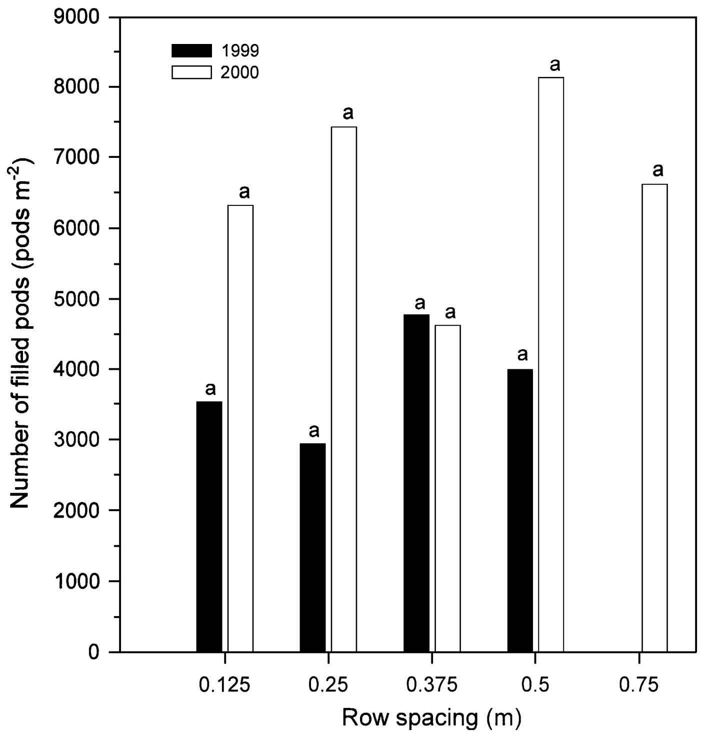

Fig. 1. Effect of row spacing on the number of filled pods per square meter land area. Within each year, mean values followed by the same letter are not significantly different at the $P = 0.1$ level.

across row spacing treatments (Fig. 1). This may explain the lack of yield response with increased row spacing (Table 1). However, confounding effects due to plant density cannot be eliminated completely. Grafton et al. (1988) showed that the number of pods per plant for indeterminate pinto bean (*Phaseolus vulgaris* L.) increased with decreasing plant density, but showed little response with increased row spacing. Bennett et al. (1977) also showed for several dry bean cultivars that pod numbers per plant decrease with increased plant population density. In our study, greater plant densities in the narrow row treatments also possibly could have led to less branching and thus fewer pods per plant than for the 0.50 and 0.75 m spacings.

High plant densities can lead to greater inter-plant competition for available environmental resources (Adams, 1967). Larger plants, as observed on wider row spacings in the present study, may have resulted from more efficient light use. Fig. 2 shows LAI for row spacing treatments on 19 July when plants were beginning to flower and 16 August during the time of seed-set in 2000. By 19 July, canopy closure already had occurred in the 0.125, 0.25, and 0.375 m plots (field observation) and LAI was at a maximum for the 0.125 and 0.25 m row spacing treatments (Fig. 2). By 16 August there was no difference in LAI across treatments except for the 0.75 m spacing, which was slightly but significantly lower (Fig. 2). Under narrow row spacing, particularly the 0.125 and 0.25 m treatments, inter-plant shading likely occurred during late vegetative growth and early anthesis. This might explain the reduced branching and hence fewer filled pods per plant for narrow row-grown *Cuphea*. Similarly, soybean (Weber et al., 1966) and dry bean (Bennett et al., 1977) also show reduced branch numbers when inter-plant spacing is decreased.

As indicated by LAI data (Fig. 2), it is likely that plants grown on wider rows intercepted more light per plant during much of their life cycle than those grown in narrow rows. Greater light capture coupled with less inter-plant competition may have allowed these plants

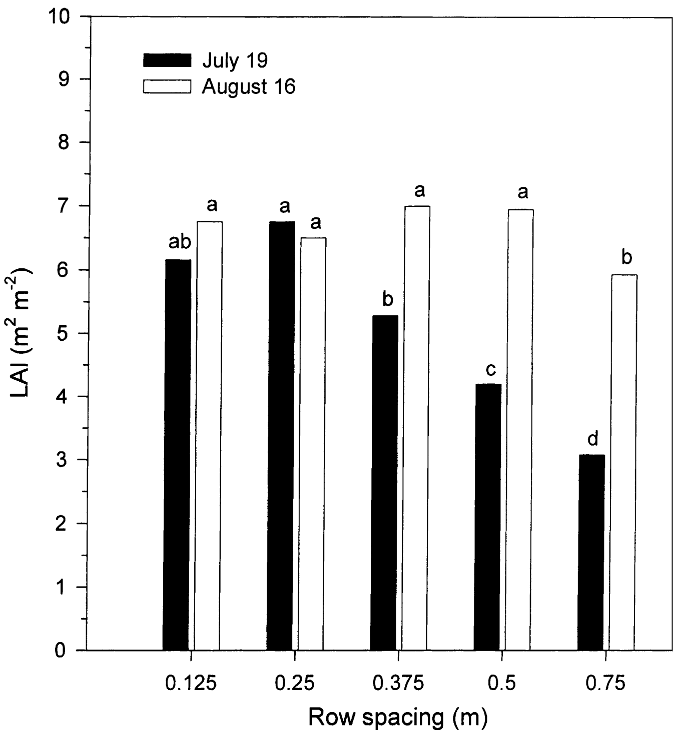

Fig. 2. Effect of row spacing on LAI during 2000. Within each date measured, mean values followed by the same letter are not significantly different at the $P = 0.1$ level.

to utilize photosynthate more efficiently. For soybean, whose growth habit is similar to *Cuphea*, highest yields under various spacial patterns often are associated with accelerated growth rate during vegetative and early reproductive phase (Parvez et al., 1989; Bullock et al., 1998). Efficient light capture and use by plants is believed to be a major determinant of this response (Board and Harville, 1992). On a per plant basis, all other factors being equal, greater light interception leads to larger plants, and as Duncan (1986) notes this often translates into greater seed yield per plant. A final noteworthy result from this study is that seed size was not affected by row spacing (Table 1).

4. Conclusion

The potential for producing *Cuphea* as a row crop in the northern Corn Belt region appears favorable. In the present study, *Cuphea* lacked yield response to inter-row spacing on an area basis due to yield compensation in wider rows (i.e., greater number of filled pods per plant) resulting from greater branching. Results indicate that greater branching of plants grown on wide rows was likely due to a combination of greater light capture and less inter-plant competition. Because of its indeterminate growth habit, *Cuphea* may be more responsive to plant population density (i.e., intra-plant spacing) than inter-row spacing.

Effective chemical broadleaf weed control for *Cuphea* is lacking. Therefore, at present, wide-row-culture of *Cuphea*, allowing for mechanical weed control, is the favored practice. However, further work is necessary for adjustments of plant spacing to optimize seed yield. Currently, we are addressing the issues of optimum plant population density for seed yield and light use efficiency of *Cuphea*.

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